Animal Care on Neurolab

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ABSTRACT

The Neurolab mission carried rats, mice, snails, crickets, and two kinds of fish into space. This was the largest variety of living things ever flown on a space mission. On the whole, the experimental animals were healthy during the flight, but there was a notable exception. Although all the mice, adult rats, and most of the young (14 days old at launch) rats did well, more than half of the youngest rats, eight days old at launch, died in the Research Animal Holding Facility inflight. Several factors contributed to this, including housing design and inadequate monitoring capability. An understanding of why this occurred is important for the planning of future space missions.

INTRODUCTION

The Neurolab mission had an ambitious animal research program. The mission carried rats, mice, two kinds of fish, snails, and crickets to accomplish the research objectives. Both adult rats and two groups of young rats (eight days old at launch and 14 days old at launch) were flown. Habitats were provided to sustain the animals. Two different types of animal habitats were available for rats and mice: the Research Animal Holding Facility (RAHF) and the animal enclosure modules (AEM). Oyster toadfish were flown in the vestibular function experimental unit (VFEU) and swordtail fish were housed in the closed ecological biology and aquatic system (CEBAS). The CEBAS also contained snails. Crickets were housed in an incubator (BOTEX) that included a centrifuge to provide artificial gravity for a control group of crickets. The fish, snails, and crickets were not removed from their habitats inflight and the hardware sustaining them required minimal crew intervention, so they will not be discussed in this report. Most of the crew activity was devoted to the rats and mice.

The crew included a veterinarian, Rick Linnehan, D.V.M, who provided and supervised care to the animals inflight, and a veterinarian as an alternate payload specialist, Alex Dunlap, D.V.M., who communicated with the crew during the flight. A veterinary kit was onboard that provided treatment capability for minor problems. Animal checks were incorporated into the timeline. Most experimental procedures were carried out in the general-purpose workstation (GPWS). The GPWS provided a contained space where animals could be removed from their cages and where a variety of experimental equipment could be deployed and used (anesthetics, fixatives, surgical instruments, infusion pumps, work platforms, etc.).

The adult rats, the 14-day-old (at launch) rats, and the mice all did well on the mission. The youngest rats (eight days old at launch), however, had significant problems. More than 50% of these rats died on the flight. An investigation into this revealed that inadequate housing and the inability to provide effective monitoring contributed to this high mortality. The mortality was probably not a unique physiological effect of weightlessness, since rats of this age had flown successfully prior to Neurolab (although in a different kind of housing). For the future, cage designs for spaceflight should incorporate surfaces that allow for easy three-dimensional navigation and reliable ways to monitor the animals.

HOUSING

Research Animal Holding Facility (RAHF)

The RAHF is a temperature- and humidity-controlled facility for housing rodents (Figure 1). Light levels and lighting duration also can be adjusted. The RAHF consists of 12 cages, each of which can house two adult rats, or one mother rat and eight young. The cages each have individual feeding alcoves and watering lixits to provide food and water, respectively. Waste is controlled by airflow that enters the cage on one side and moves the waste to a tray on the opposite side. The individual cage walls were aluminum, and not all surfaces had areas that the rats could grasp. A diagram of the RAHF is shown in Figure 1.

The food system on the RAHF includes a method to measure food consumption. By pulling on a measuring tape, a rough estimate of the amount of food bar consumed can be obtained. The water system also sends data to the ground on how many times water moves through the lixit. Each cage also can detect activity, based on a light sensor. When an animal moves by the sensor, the light beam is interrupted, and this provides a rough measure of activity. Experience on previous spaceflights, however, had shown that weightless objects floating in the cage also trigger the sensor and render the data useless. As a result, the activity data were not used on Neurolab.

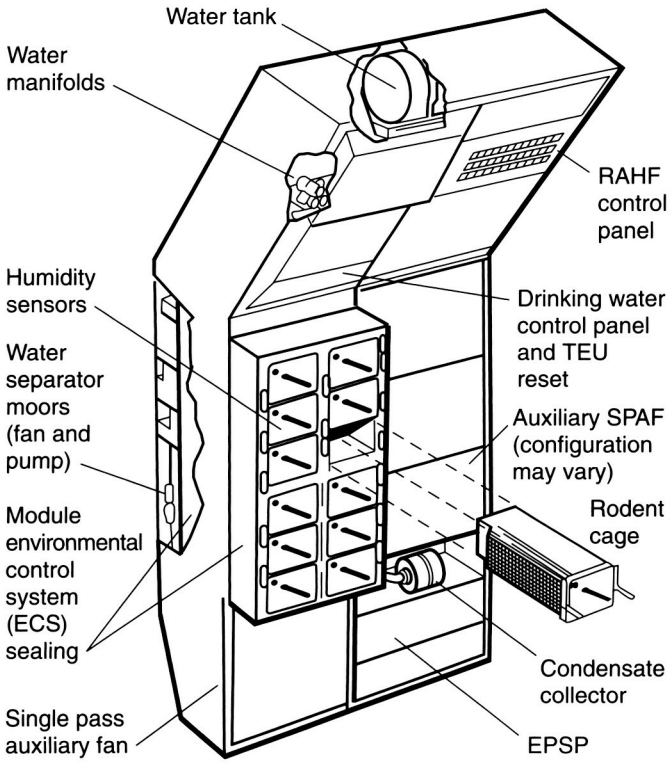

Figure 1. The Research Animal Holding Facility consisted of 12 cages that could house two adult rats or one mother rat and eight young rats. The various systems on the RAHF provided a temperature- and humidity-controlled environment. Water outlets in the cages (lixits) provided water to the rats when the touched the lixit. Food bars were dispensed through a mechanical system.

Each cage module also has a small window. From this window, however, the rear cage cannot be seen, and the view of the front cage is constrained.

Animal Enclosure Module (AEM)

The AEMs are animal habitats designed to fit in a middeck-sized locker (Figure 2). Instead of cages, the animals are housed as a group in an open area. Food is available at the center of the AEM, and water is provided through lixits. The cover of AEM is clear plastic, so the animals can be seen easily. The AEMs were not originally designed to allow for animals to be taken out during the flight. For Neurolab, a special attachment was built that allowed the crew to take animals from and return them to the AEM. A picture of the AEM is shown in Figure 2. On the NIH.R3 Space Shuttle mission, young rats (eight days old at launch) were flown successfully in an AEM.

Comparison of the two housing methods

The RAHF provided individual cages and tight control of environmental parameters. Also, individual food and water consumption could be measured. The RAHF had the disadvantage that the animals could not be easily seen. The AEMs provided group, rather than individual housing, and so did not provide individual measurements of food and water consumption. The rats, however, could be easily seen through the plastic lid. They could also move about easily on the wire surfaces that lined the AEM.

Treatment capabilities

The veterinary kit on board provided fluids for dehydration and antibiotics (enrofloxacin) in case of infection. Also, the kit had Nembutal for euthanasia.

Inflight loss of young (eight days old at launch) rats[1]

The most significant animal issue on the flight was the unexpected death inflight of more than 50% of the youngest rats (eight days old at launch). This occurred in the RAHF located in Spacelab rack 3 (RAHF3).

Twelve Sprague-Dawley female rats, each with a litter of eight young rats, were loaded into RAHF 3 cages 40 hours before launch. Integration of the cages into the RAHF was normal and data indicated that the RAHF was operating as planned. Lixit (water) counts demonstrated that the mothers were consuming water as expected, and this continued over the next two days while STS-90 remained on the launchpad awaiting launch. After launch while on orbit, telemetered data demonstrated normal water consumption and RAHF operation. During the prelaunch period, the Spacelab atmospheric carbon dioxide partial pressure gradually increased to 13 mmHg

[1]The authors would like to acknowledge the white paper written by Louis Ostrach at NASA-HQ, which also included information about this event that was used in this report.

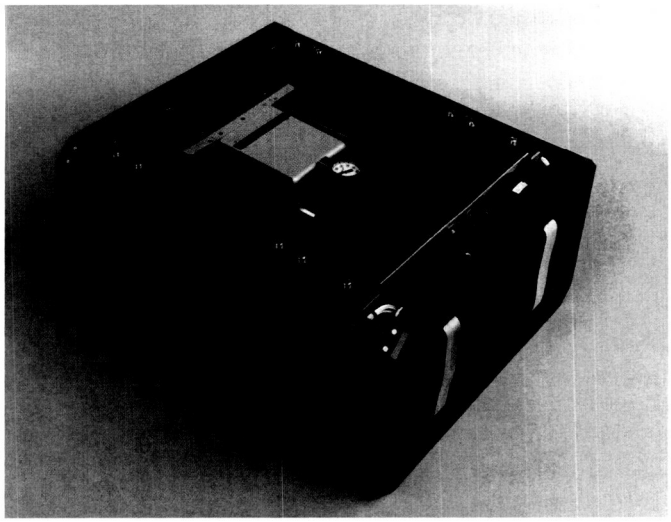

Figure 2. The animal enclosure module provided group housing for the rats. Food was available on exposed food bars in the center of the module and water was available through lixits. The clear cover allowed for the rats to be easily seen.

(1.7%) and then dropped precipitously to ~2 mmHg after Spacelab activation. Based on limited data available in the literature, it is unlikely that these conditions would have affected either the mothers or the young rats.

On FD1, FD2, and FD6, the crew performed the scheduled feeder tape measurements and visual checks on RAHF3. The feeder tape measurements showed that food was being consumed; however, visual checks on the rats were problematic. Rat health could not be accurately assessed through the RAHF windows (this was a known problem from experience on the SLS-2 flight). The windows offered a partial view of the front cage; this view worsened over time as the inside of the windows became coated with particles. Also, since the rats often spent their time in the rear of the cages, usually they could not be seen.

The first planned activity involving the RAHF3 rats was scheduled for FD6. Due to changes in the timeline, however, testing on these animals was postponed and no RAHF3 cages were pulled on that day. The first time a cage was removed from RAHF3 was on FD8. At this time, it was found that two of the eight young rats in that cage had died. Since this was unanticipated, the remaining cages were pulled and an inventory of all the rats in RAHF3 was conducted. In 11 of the 12 cages, some young rats (ranging from one to six per cage) had died. Upon opening each cage, the crew rated whether the remaining rats appeared sick or healthy. Out of the 96 young rats that were launched, 38 had died and 19 appeared sick. The sick rats were given subcutaneous fluids and antibiotics (enrofloxacin). The fluid mix was 0.113 mg enrofloxacin/mL fluid. One mL of this solution was given to each sick rat. Despite this, 12 sick rats were clearly unable to survive and were euthanized. The young rats were redistributed to give the sick rats more opportunity to feed from a mother rat. The status of the rats and the work that had been done with them was put in a spreadsheet and sent to the ground. A private veterinary conference was held to discuss the findings and determine the best plan of action. At the end of FD8, there were 46 young rats remaining in RAHF3.

On each subsequent day, the crew pulled every cage in RAHF3, brought it to the workstation, and checked the health of all the rats. Some rats worsened and were euthanized. Most rats improved and those that appeared sick where given fluids and enrofloxacin. Some rats also received dilute Gatorade and were warmed with heated fluid bags. Spreadsheets were completed each evening to send to the ground to help with replanning. On FD9 there were 44 rats remaining, 40 on FD10, 39 on FD12 and 38 on FD13.

By FD13 the situation in RAHF3 had stabilized. Gel packs (water-containing gel) that the rodents could use to get water in addition to the lixits had been added to the cages. There was some evidence that the mothers were neglecting the young in some cages, and the rats were placed in cages where the other young rodents were doing well.

After landing, the most notable finding was that the rats and cages were soaking wet and the neonates were hypothermic. Two more young rats had died, and one had to be euthanized.

Issues and Recommendations

Ultimately, a group of the young rats did survive and a subset of them appeared healthy throughout. Overall, however, the mortality rate was extremely high, and this has to be taken into account when reviewing the results from the development experiments involving the youngest rats on Neurolab. The problems that occurred with the young rats highlight several issues that can be useful for future space research.

Housing

Young rats had flown in space prior to Neurolab and had done reasonably well. The NIH.R3 Shuttle flight test established a 90% survival in two litters of 10 young rats eight days old at launch. These animals, however, still weighed ~25% less than age-matched AEM ground controls and ~30% less than vivarium controls after landing. Also, this flight used AEMs for housing and not the RAHF. The RAHF cage, which does not have the wire surfaces that the AEM does, would not provide the rats with as many surfaces to grasp and use for navigation. Since in weightlessness the rat can float in three dimensions, all walls need to have surfaces that the rats can grasp. This is especially important because eight-day-old rats have their eyes closed and depend upon the mother for food. If they were to become detached from the mother and not have a way to navigate back, they could end up becoming dehydrated and malnourished. It is possible that this was one factor at work on the Neurolab mission. Also, a mission-length test with young rats using the exact RAHF cages was not performed prior to the mission (the RAHF had flown successfully several times prior to Neurolab). For future missions, the housing should be tested extensively to make sure that it could provide adequate support.

Monitoring capability

The problems on Neurolab might have been averted or minimized if the crew or ground had had some indication of problems within the cages. As it turned out, however, none of the monitoring systems were adequate to detect the problems within the cages. Lixit counts remained normal in all cages. The food bar measurements were consistent with previous experience. The windows were not adequate to allow the crew to see the problems in the cages, and the activity monitoring system had been shown prior to Neurolab to be unreliable. For future habitats, the ability to have reliable monitoring of animals is critical.

Veterinary care

The crew on Neurolab had the ability to remove cages and work with the animals in the GPWS. This capability was very important to salvage the experiments and stop the decline in the health of the rats in RAHF3. The veterinary kit that was initially proposed for the mission had minimal capabilities. The crew was able to add extra items to the kit preflight, such as the fluids and antibiotics, which subsequently were used on the mission. This experience demonstrated the importance of having the crew trained in the appropriate actions to take, involved in decisions on kit contents, and having the necessary tools on board.

CONCLUSION

The Neurolab mission had a significant animal care problem in space. The problem was unanticipated, and seemed unlikely based on the testing that preceded the mission. The crisis highlighted the importance of detailed preflight hardware testing, and on the provision of dependable monitoring systems for animals. Also, the ability of the crew to treat the animals inflight was an important factor in stabilizing the situation. In the future, it is likely that young rats can be flown successfully if the hardware is robust, reliable monitoring is present, and the crew can intervene if a problem arises.